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Full Length Research Paper

Influence of technological treatments on bacterial communities in tilapia *(Oreochromis niloticus)* as determined by 16S rDNA fingerprinting using polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE)

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Food quality and safety are major concern among consumers throughout the world in the context of globalization. Hence, the origin and the history of a food item are of prime interest when food quality is questioned. Precise determination of contamination source relies on the use of efficient and reliable methods. This study was carried out to assess the microbial ecology of fish upon technological treatments using 16S rDNA polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) fingerprinting. Samples of tilapia from Montpellier (South-east of France) and Yagoua (far north of Cameroon) were used for this purpose. The technological treatments applied on fillets were marinade, drying, smoking and deep-freezing. When the 16S rDNA profiles were analysed by multivariate analysis, distinct microbial communities were detected. The band profiles of fish bacteria after each treatment were different and specific. Technological treatments applied on fillets from Montpellier did not have an effect on the biological markers present on the fillets. These bands could be used as specific markers for this region. One of the treatments, the marinade applied on the samples of Yagoua induced the disappearance of some bands on the DGGE profile. In spite of treatment applied on samples, it is possible to recover the geographical origin by using DNA of the bacterial community in fish even if it was treated.

Key words: Traceability, tilapia, polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE), bacterial community, technological treatments.

INTRODUCTION

Tilapia which belongs to the family of Cichlidae and order of perciformes is a vegetarian fish. It is mostly appreciated for the quality and the taste of its flesh, the tolerance to different environments and the resistance to illnesses. The interest in tilapia explains the tremendous increase of the production in the last decade: 400 000 tons in 1990 and 1 800 000 tons in 2004. However, it has been shown that the freshly collected fish is contaminated by high concentrations of microorganisms: 10^2 to 10^7 CFU/cm² of skin surface (Liston, 1980) and 10^3 to 10^9 CFU/g of gills or intestines (Shewan, 1962). The world, particularly Europe has undergone many food crises, among these are listerioses, bovine spongiform

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encephalopathy (BSE) and salmonelloses (Montet et al., 2004). Food quality and safety are major concern among consumers throughout the world in the context of globalization. Hence, the origin and the history of a food item are of prime interest when food quality is questioned. The determination of geographical origin is one demand of the traceability of import-export products. One hypothesis of tracing the source of a product is by analysing it in a global way the bacterial communities on the food sample. The predominant bacterial flora would permit the determination of the capture area, production process or sanitary or hygienic conditions during post harvest operations (Montet et al., 2004).

Many studies have shown that there is a link between aquatic micro-organisms and those of the fish. So, water composition, temperature and weather conditions can influence the bacterial community in fish (Austin and Austin, 1999; Wong et al., 1999; Jallabert et al., 2000; De Souza and Silva-Souza, 2001).

Traceability is the capacity to find the history, use and origin of a food by registered methods (ACTA-ACTIA, 1998). Genetic traceability is realised by the characterization of DNA, since the microbial flora of aquatic products reflects those of the environment (Shewan, 1977; Horsley, 1977). Traceability uses recent techniques of molecular genetics which permit to visualize some characteristics of hereditary patrimony of individuals. Among the techniques of microbial characterization in foods, there are methods of independent culture that provide a profile representing genetic diversity of a specific environment (Giovanni et al., 1990). Several techniques of DNA analysis are used and all are based on the amplification by polymerase chain reaction (PCR) from the DNA of ecosystem. The mixture of PCR products is then analyzed by various techniques of electrophoresis, either by methods based on the analysis of size fragments [randomly amplified polymorphic DNA (RADP), amplified ribosomal DNA restriction analysis (ARDRA), restriction fragments length polymorphism (RFLP) and terminal RFLP (T-RFLP)] or by methods based on the sequence of fragments [single strand chain polymorphism (SSCP), temperature gradient gel electrophoresis (TGGE)/ denaturing gradient gel electrophoresis DGGE)].

Polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) is a method that combines two stages: firstly a stage of amplification using a unique pair of primers by PCR and a second stage of electrophoresis in acrylamide gel in denaturing conditions. Separation of PCR products in DGGE is based on the decrease of the electrophoretic mobility of partially melted doubled-stranded DNA molecule in polyacrylamide gels containing a linear gradient like formamide and urea at 60°C. Molecules with different sequences will have a different melting behaviour and will stop migrating at different position in the gel (Muyzer et al., 1993; Leesing, 2005). PCR-DGGE method presents incontestable advantages; it is rapid and done without germ culture step in Petri dishes.

The bacterial species differentiation is based on the differential migration in gel during the DGGE according to the percentage of guanine + cytosine. The result obtained is expressed as a code bar in which every band corresponds to a micro-organism of the microbial community (Muyzer et al., 1993; Muyzer et al., 1996).

In previous studies, Leesing (2005), Le Nguyen et al. (2007), Montet et al. (2008) and Tatsadjieu et al. (2009) used this technique on fresh fishes and they found out that the band pattern of the bacterial communities isolated from fresh fish obtained by PCR-DGGE was strongly linked to the microbial environment of the fish. They showed also that this technique could be applied to differentiate geographical location.

The fish can be cooked immediately or treated before cooking. In order to preserve the quality and prolong the shelf life of fresh fish, some technological treatments are used. Among these ones are deep-freezing, drying and smoking. Marinade is used in most cases to tenderize and flavour the fillet. We choose these four treatments because they are the most used all over the world.

The purpose of our study was to apply the PCR-DGGE method in analyzing the bacterial community on dried, marinated, smoked, refrigerated and deep-frozen fishes in order to determine if technological treatments have an effect on microbial markers of geographical origin.

MATERIALS AND METHODS

Fish sample collection and treatment

The biological material used during this analysis was tilapia (*Oreochromis niloticus*) collected in the Logone River of Yagoua (July, 2009) in Cameroon where the temperature average was 30.5°C and in an experimental pond of the Cirad of Montpellier (August, 2009); in this case, the fishes were bred in tropical conditions (temperature up to 23°C). At least four fishes were collected from the two different locations. The samples were taken from the river and/or the pound and transferred to storage sterile bags. The samples were maintained on ice and transported to the laboratory. On each fish sample, two fillets were cut off and shared in two lots. The first lot constituted of fillets to be refrigerated and the second lot was treated. Each part of the treated fillet and non treated were put in separate sealed plastic bags, then kept frozen at -20°C until further analysis. Four types of treatments were applied to these fillets: marinade, drying, deep-freezing and smoking (only for Yagoua samples). During the marinade, fillets were immersed in acetic acid 4% for 30 min. The samples were dried in an oven at 105°C for 3.5 h. For deep-freezing, the fillets were placed in an enclosed and ventilated space at -40°C for 30 min. Smoking was carried out for 24 h with a mixture of fresh avocado leaves laid down on embers.

Total DNA extraction

The DNA extraction was based on the method of Ampe et al. (1999) and Leesing (2005) but modified and optimised. For all the samples, 2 g of treated fillet was introduced in a sterile stomacher bag and ground with microbiological stomacher 3500 grinder for 3 min in sterile peptone water. 6 ml of the resulting mixture was

poured in four Eppendhorf tubes for DNA extraction.

The previous mixture obtained was centrifuged at 10000 rpm/min (Biofugopico Heraeus, Germany) at 4°C for 10 min. To the pellet obtained, 100 µL of buffer tris/ethylenediaminetetraacetic acid (TE) (Promega, France), 100 µL of lysozyme solution (25 mg/mL, Eurobio, France) and 50 µL of proteinase K solution (20 mg/mL, Eurobio, France) were added. Samples were mixed in vortex for 1 min and incubated at 42°C for 20 min. 50 µL of 20% sodium dodecyl sulphate (SDS) was added and the mixture was incubated at 42°C for 20 min. 400 µL of mixed alkyl trimethyl ammonium bromide (MATAB) 2% was added to hydrolysis products (65°C/10 min). After cellular lyses, a volume of phenol/chloroform/isoamyl alcohol (25/24/1, Carlo Erba, France) was added and the mixture was centrifuged at 10000 rpm/min for 10 min to allow the DNA extraction. A volume of chloroform/isoamyl alcohol (24/1) mixture was added and centrifuged at 10 000 rpm/min for 10 min in order to purify the DNA and to remove the remaining phenol. Proteins and the remaining polysaccharides in the aqueous phase were recovered at the interface with the organic phase after centrifugation at 10000 rpm/min for 10 min. The total DNA was precipitated with isopropanol (-20°C) followed by centrifugation. A volume of ethanol 70% was added to remove water around the DNA molecule. The DNA obtained was suspended in 50 µL of TE and stored at -20°C.

DNA amplification by polymerase chain reaction (PCR)

The 16S rDNA were specifically amplified by PCR using bacterial couple of primers described by Muyzer et al. (1993): Gc338f (5'CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGG GGAC

TCCTACGGGAGGCAGCAG, Sigma, France) and 518r (5'ATTACCGCGGCTGCTGG, Sigma, France). Amplification of the V3 variable region of bacterial communities' 16S rDNA of fishes was realized using a guanine + cytosine (GC) clamp of 40 nucleotides. Each mixture (final volume of 50 µL) contained about 100 ng of template DNA. All the primers were at 0.2 µM, all the deoxyribonucleotide triphosphate (dNTPs) at 200 µM, 1.5 mM $MgCl_2$, 5 µL of 10X of Taq buffer $MgCl_2$ free (Promega, France) and 5U of Taq polymerase (Promega, France).

During PCR reaction, non-specific hybridizations could be created; these hybridizations are due to complementary micro-sequences. To improve the specificity of the reaction, a ''touchdown'' PCR was performed according to the protocol of Diez et al. (2001). An initial denaturation at 94°C for 1 min and 10 touchdown cycles of denaturation at 94°C for 1 min, then annealing at 65°C (with an increasing temperature 1°C per cycle) for 1 min and extension at 72°C for 3 min followed by 20 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 3 min.

PCR products (5 µL) were then analyzed by conventional electrophoresis in 2% (w/v) agarose gel with 1X tris-acetate EDTA (TAE) buffer. 5 µL of products and 2 µL of stain (Bromophenol Blue) were loaded in wells. The migration lasted for 60 min at 100 V and was quantified with DNA mass ladder of 100 pb (Promega G2101 France). At the end of migration, the agarose gel was immersed in ethidium bromide (50 mg/L) (ETB: Promega H5041, France) for 15 min (Ampe et al., 1999), rinsed with distilled water for 30 min and then, observed on ultraviolet (UV) transilluminator using Gel Smart 7.3 system (Clara Vision, Les Ulis, France).

Denaturing gradient gel electrophoresis (DGGE)

PCR products were analyzed by DGGE using the DCodeTM detection system (Bio-Rad laboratory, Hercules, USA). Method first described by Muyzer et al. (1993) and improved by Leesing (2005). Samples containing approximately equal quantities of amplicons were loaded into 8% w/v polyacrylamide gel (Promega, France) with a denaturing gradient urea formamide spreading 30 to 60% (100% corresponding to 7 M of urea and 40% v/v, formamide, Promega, France). Volumes of PCR products used were 10 and 20 µL. Electrophoresis was performed at 60°C in TAE buffer (2 M tris-acetate, 0.05 M EDTA, pH 8.3) at 20 V for 10 min and then 80 V for 12 h. After electrophoresis, the gels were stained with ETB 50 mg/L for 30 min and rinsed in distilled water for 20 min. The gels were photographed on a UV transilluminator using Gel Smart 7.3 system (Clara Vision, Les Ulis, France).

Gels analysis

Individual lanes of the gel images were straightened and aligned using ImageQuant TL software vision 2003 (Amesham Biosciences, USA). Gels were analyzed by the software Picture As for TL (Picture Analysis Software, 2003). The permits automatically situated bands on gels and generated their migration fronts (Rf) corresponding to volumes of the band. Banding patterns were standardised with the two reference patterns: *Escherichia coli* and *Lactobacillus plantarum* DNA amplicons included in all the gels. This software permitted the identification of the bands and their relative position compared with the standard patterns. The generated bands represent different species of bacteria in a population. On the gel, an individual discrete band refers to a unique phylotype (Muyzer et al., 1996). The DGGE were manually scored as presence of band (1) or by absence of band (0) independent of the intensity. Patterns were compared using the Dice similarity coefficient (S_D) calculated according to the following formula given by Heyndrickx et al. (1996):

$$S_D = 2Nab / (Na+Nb)$$

Where Nab represents the number of bands common to sample A and B, Na and Nb represent the number of bands detected in sample A and B, respectively. Similarities index were expressed within a range of 0 (completely dissimilar) to 1 (perfectly similar). Dendograms were constructed using Statgraphics plus version 5 software (Sigma plus, France). Similarities in community structure were determinate using the cluster analysis with Euclidian distance measure.

RESULTS

Effect of technological treatments on French fish (Montpellier, France)

The samples were collected in an experimental pound of Cirad in Montpellier. The fillets had undergone marinade, deep-freezing and drying as treatments. Non treated samples in this case were represented by the refrigerated fillets. Amplifications were been realized on the total DNA extracted from fish fillets by using a couple of primers Gc 338f and 518r. On the verification gel obtained, the bands observed were between 298 and 220 pb of the molecular leader. For the three treatments realized, there were differences in band intensity; so, intensity of patterns 2 and 9 were of lower intensity than the others (Figure 1). The PCR-DGGE patterns of three replicates for each treatment revealed the presence of 7 to 14 visible and distinct bands of bacteria in fish (Figure 2 and Table 1).

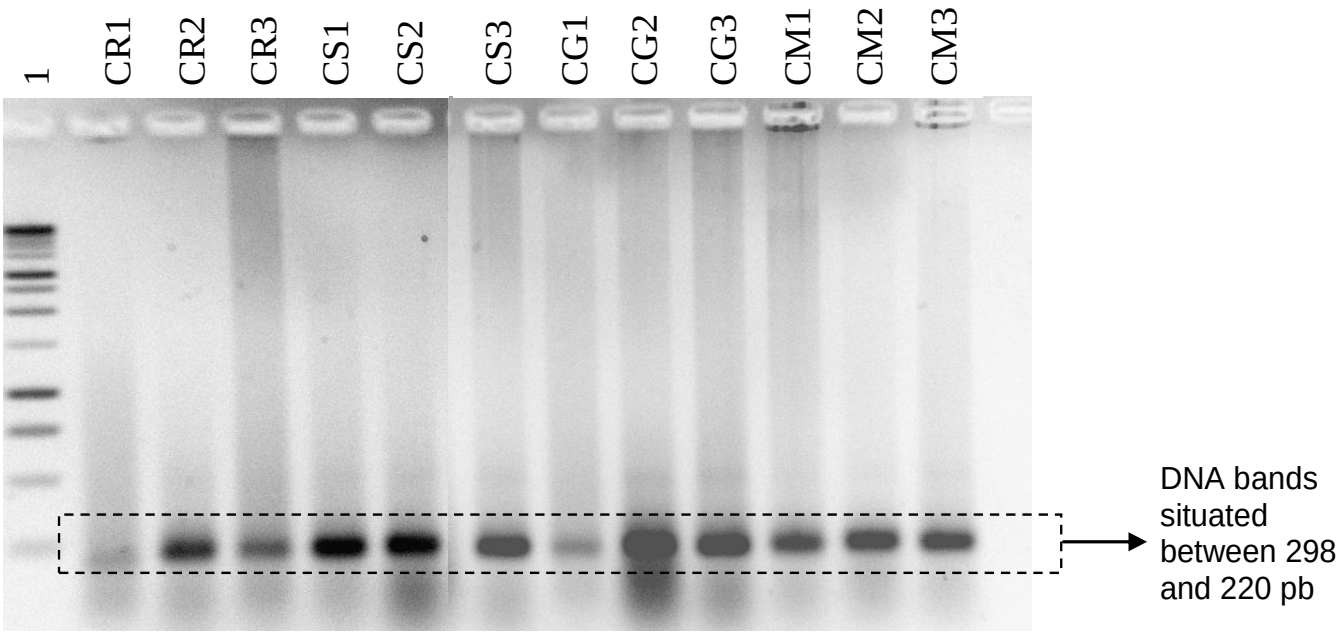

Figure 1. Verification gel of PCR products from the total bacterial DNA extracted from French fish. 1, DNA molecular leader; CR, refrigerated samples; CS, dried samples; CG, deep-frozen samples; CM, marinated samples. PCR, Polymerase chain reaction.

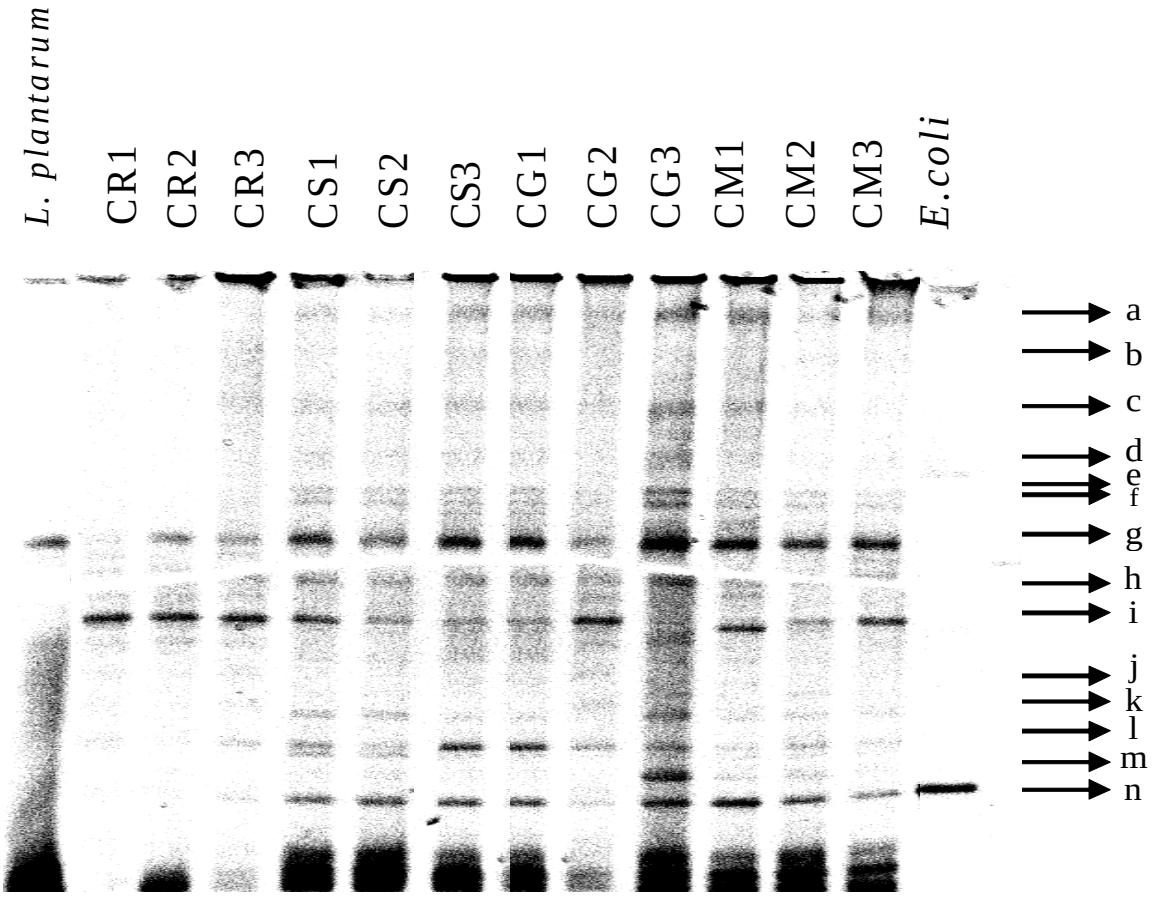

Figure 2. PCR-DGGE of 16S rDNA banding profiles of treated fish from Montpellier (France). CR, Refrigerated samples; CS, dried samples; CG, deep-frozen samples; CM, marinated samples. PCR-DGGE, Polymerase chain reaction-denaturing gradient gel electrophoresis.

Table 1. Rf of migration observed on DGGE gels of French fish (Montpellier, France).

Rf	Lp	CR1	CR2	CR3	CS1	CS2	CS3	CG1	CG2	CG3	CM1	CM2	CM3	Ec
0.11	0	0	0	0	1	1	1	1	1	1	1	1	1	0
0.21	0	0	0	1	1	1	1	1	1	1	1	1	1	0
0.25	0	0	0	0	1	1	1	1	0	1	1	1	1	0
0.36	0	0	0	0	1	1	1	1	1	1	1	1	1	0
0.43	1	1	1	1	1	1	1	1	1	1	1	1	1	0
0.47	0	1	1	1	1	1	1	1	1	1	1	1	1	0
0.50	0	1	1	1	1	1	1	1	1	1	1	1	1	0
0.54	0	1	1	1	0	0	0	1	1	1	0	0	0	0
0.58	0	0	0	0	1	1	1	0	0	0	1	1	1	0
0.65	0	1	1	1	1	1	1	1	1	1	1	1	1	0
0.68	0	1	1	1	1	1	1	1	1	1	1	1	1	0
0.72	0	1	1	1	1	1	1	1	1	1	1	1	1	1
0.83	0	0	0	0	1	1	1	1	1	1	1	1	1	0

Lp, *Lactobacillus plantarum*; Ec, *Escherichia coli*; CR, refrigerated samples; CS, dried samples; CG, deep-frozen samples; CM, marinated samples; Rf, migration fronts; DGGE, denaturing gradient gel electrophoresis.

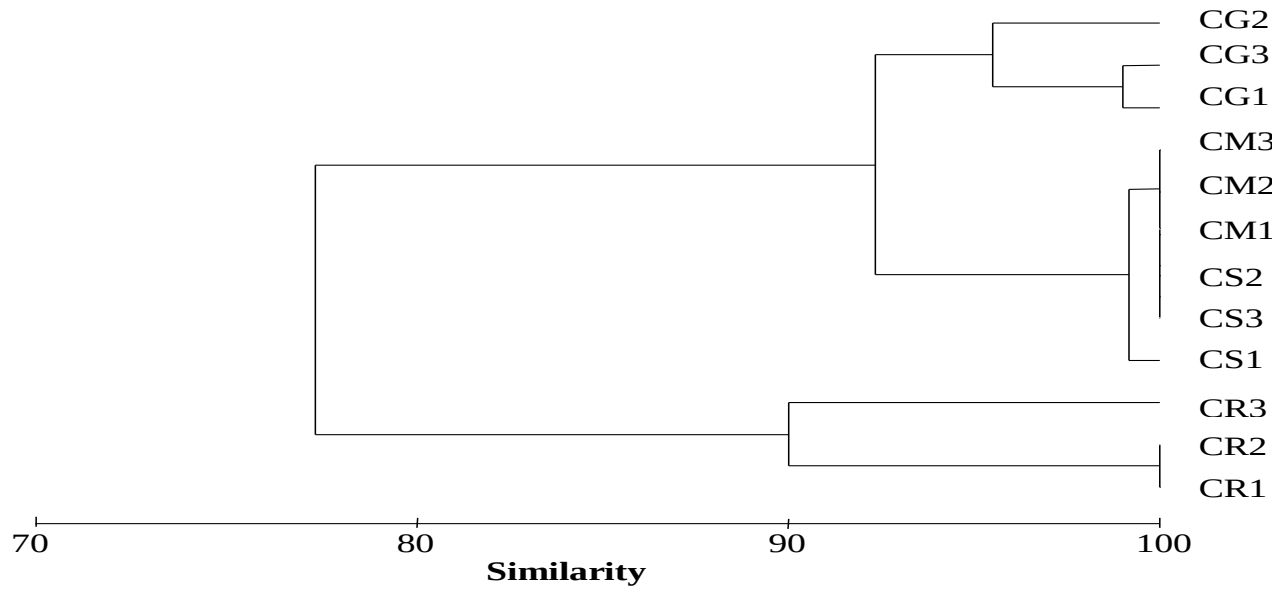

Figure 3. Cluster analysis of 16S rDNA banding profile for treated fish bacterial communities from Montpellier (France). CR, Refrigerated samples; CS, dried samples; CG, deep-frozen samples; CM, marinated samples.

The references *E. coli* and *L. plantarum* patterns confirmed a good migration in DGGE. There were about six bands (g, h, i, l, m and n) common to all the treated samples (Figure 2). The pattern obtained for the bacterial community for the three replicates of the same treatment was almost similar. High similarity was also observed between marinated and dried samples. The statistical analysis of DGGE gel patterns shows the community similarity among treated and non treated fish samples (Figure 3). At 90% similarity level, two main clusters were observed: the first cluster was constituted of refrigerated samples while; the second cluster included marinated, dried and deep-frozen samples. The bacterial communities of marinated, deep-frozen and dried samples were closely related. These results show also that dried, marinated and deep-frozen samples were closer in term of bacteria ecology.

Effect of technological treatments on Cameroon fish

The samples were collected in the Logone River in the far north region of Cameroon. The fillets have undergone marinade, smoking and drying as treatments. Non treated

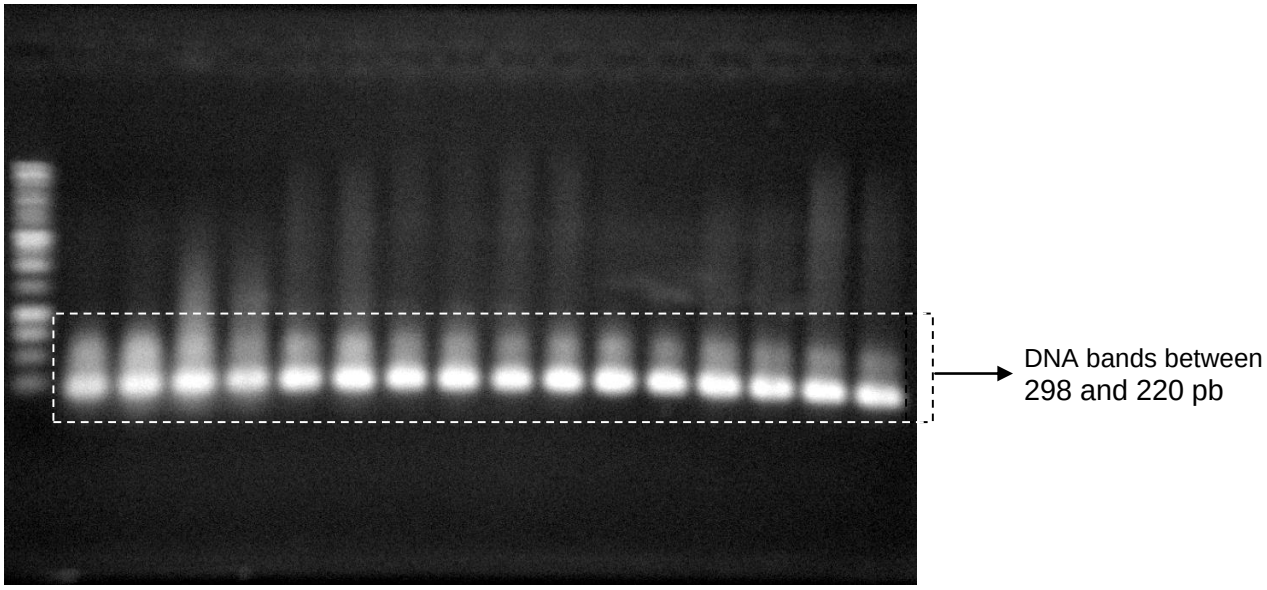

Figure 4. Verification gel of PCR products from the total bacterial DNA extracted from Yagoua treated fish. 1, DNA molecular leader; FY, smoked samples; SY, dried samples; Y, non treated samples; MY, marinated samples. PCR, Polymerase chain reaction

samples were represented by the refrigerated fillets. As for the French samples, the bands observed on the gel were between 298 and 220 pb of the standard patterns. DNA amplicons of Yagoua had a good intensity (Figure 4). The PCR-DGGE patterns of three replicates for each treatment revealed the presence of two to six visible and distinct bands of bacteria in fish (Figure 5). As for the first DGGE gel, the references *E. coli* and *L. plantarum* patterns confirmed a good migration. The great number of bands was observed on non treated samples while marinated samples presented fewer bands. However, among the treated samples of fish fillets, some bands of bacteria had disappeared. The DGGE pattern revealed distinct bands with Rf distances between 0.26 and 0.89 (Table 2). There were two bands (f and h with Rf, respectively equal to 0.48 and 0.57) common to dried, non treated and smoked samples (Figure 5). The pattern obtained for the bacterial community for the four replicates of the same treatment was almost similar. Statistical analysis of DGGE gel patterns shows a community similarity among treated and non treated fish samples (Figure 5). The dendrogram obtained (Figure 3) show that at 65% similarity level with two main clusters were formed; the first cluster included marinated samples while the second one included non treated, dried and smoked samples. At 98% level, the dendogram revealed that dried and smoked samples were closely related.

DISCUSSION

Analysis of bacterial communities in fish samples has been often investigated using culture dependant methods and culture independent methods (Spanggaard et al., 2000). There are only few published works that analyzed the bacterial communities on fish samples by PCR-DGGE methods (Spanggaard et al., 2000; Leesing, 2005; Le Nguyen et al., 2007; Montet et al., 2008; Tatsadjieu et al., 2009; Maïwore et al. 2009). These studies were carried out on fresh and non treated samples of fish.

In our study, the treatments induced some differences. The band patterns of the bacterial communities isolated from fish obtained by PCR-DGGE were strongly linked to microbial environment of the fish. The skin is directly in contact with water. That explain why there were common bands to all the treatments of the same location.

For a specific treatment, the analysis of samples (apart from the location) showed some differences in the migration pattern on the DGGE gel. However, the replicates for each treatment had almost similar DGGE pattern throughout the study. The differences in the bands profiles could be attributed to the bacterial cell wall sensibility to physical agents when the fillets were treated (Mescle and Zucca, 1996; Giraud, 1998). These differences could also be attributed to the fact that during the DNA extraction, the cellular membrane of bacteria undergo an enzymatic hydrolysis, and all microbial species do not have the same sensibility to lytic agents because of the differences in their cell wall organisation (Wilson, 1997).

During verification of PCR products, it was shown that some French samples had bands of low intensity than the others. This could be explained either by the fact that while amplification was running, PCR reaction could be

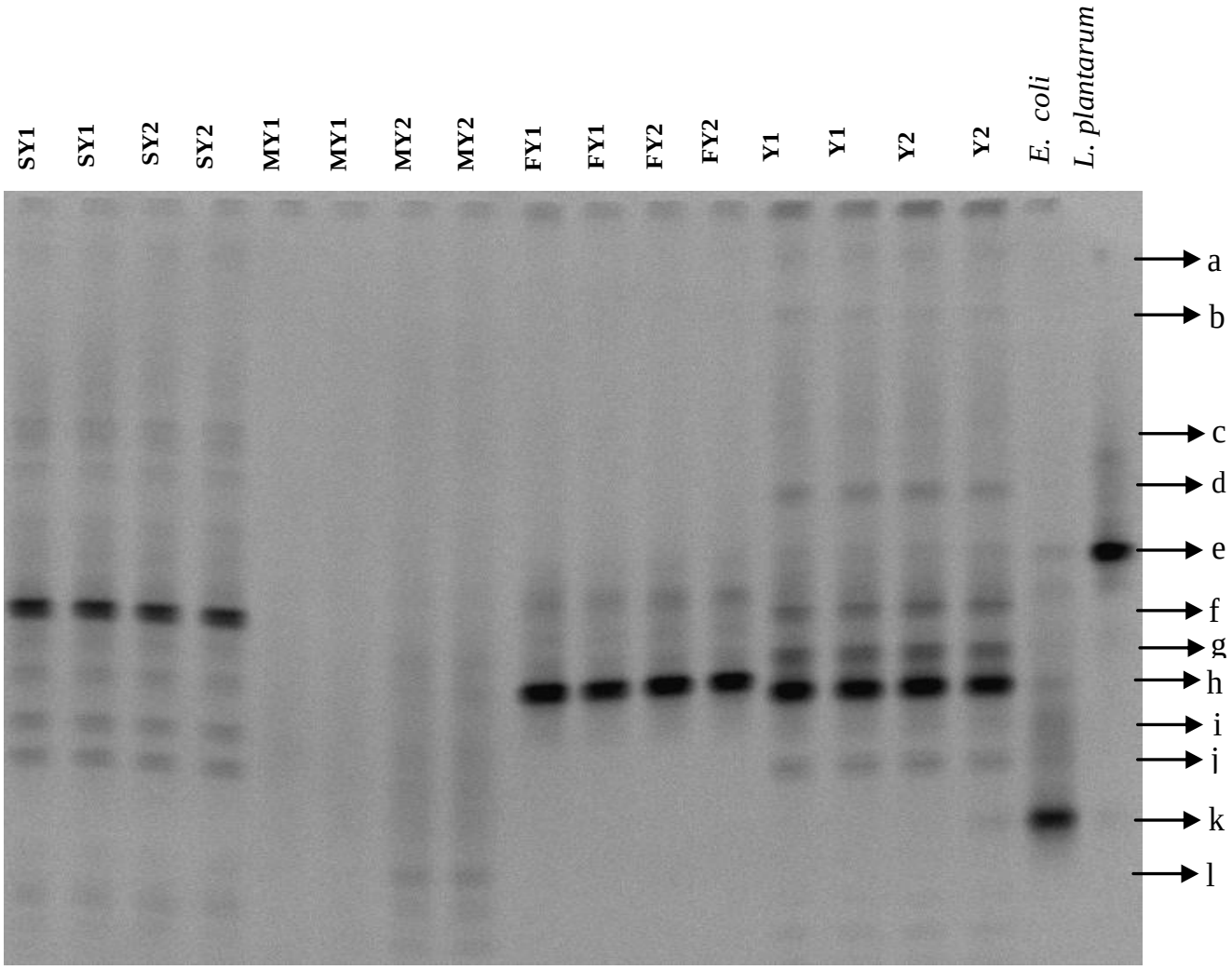

Figure 5. PCR-DGGE of 16S rDNA banding profiles of treated fish from Yagoua (Cameroon). FY, Smoked samples; SY, dried samples; Y, non treated samples; MY, marinated sample. PCR-DGGE, Polymerase chain reaction-denaturing gradient gel electrophoresis.

Table 2. Rf of migration observed on DGGE gels of Yagoua (Cameroon) fishes.

Rf	SY1	SY1	SY2	SY2	MY1	MY1	MY2	MY2	FY1	FY1	FY2	FY2	Y1	Y1	Y2	Y2	Ec	Lp
0.26	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.34	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0
0.41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
0.45	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0
0.48	1	1	1	1	0	0	0	0	0	0	0	0	1	1	1	1	0	0
0.54	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0
0.56	1	1	1	1	0	0	0	0	1	1	1	1	1	1	1	1	0	0
0.61	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.72	1	1	1	1	0	0	0	0	0	0	0	0	1	1	1	1	1	0
0.78	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
0.83	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.89	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0

Lp, *Lactobacillus plantarum*; Ec, *Escherichia coli*; FY, smoked samples; SY, dried samples; Y, non treated samples; MY, marinated samples; Rf, migration fronts; DGGE, denaturing gradient gel electrophoresis.

inhibited by impurities like polysaccharides, lipids and proteins (Wilson, 1997) or by the reduced quantity of DNA extracts injected.

Technological treatments applied to French fish sample did not have a destructive effect on bacterial communities because the main bands present on non treated samples were represented on the profiles of treated samples. Technological treatments in this case have not affected traceability markers.

Marinade applied on Cameroon fish samples induced disappearance of some bands on DGGE profile. This could be due probably to bacteria dilution in acetic acid used for technological treatment. As a result of this dilution, there was no revelation of bands after running DGGE probably because the bacterial population was less than 10^4 (Dewettinck et al., 2001; Ogier et al., 2002; Fontana et al., 2005).

Conclusion

The aim of this study was to evaluate the effect of some technological treatments on bacterial 16S DNA finger-prints of bacterial communities present on fishes samples collected from Yagoua (Cameroon) and Montpellier (France). For this study, the PCR-DGGE analysis was used. We showed that the biological markers in most cases stayed stable among the different treatments. This global technique is quicker (less than 24 h) and can be used to determine the origin of fish even if the fillets have undergone technological treatment.

REFERENCES

ACTA-ACTIA (1998). Traçabilité, guide pratique pour l'agriculture et l'industrie alimentaire. ACTA ; Paris 19 Av. du Maine, 75732 Cedex. 15: p. 80.

Ampe F, Ben Omar N, Moizan C, Wacher C, Guyot JP (1999). Polyphasic study of the spatial distribution of microorganisms in mexican pozol, a fermented maize dough, demonstrated the need for cultivation-independent methods to investigate traditional fermentations. Appl. Environ. Microbiol. 65: 5464-5473.

Austin B, Austin DA (1999). Bacterial fish pathogens. Disease of wild and farmed fish. Third edition (revises), Springer-Praxi, London. p. 457.

De Souza JA, Silva-Souza AT (2001). Bacterial community associated with fish and water from Congonhas river, Sertaneja, Parana, Brasil. Braz. Arch. Biol. Technol. 44(4): 373-381.

Dewettinck T, Hulsbosch W, Van Hege K, Top EM, Verstraete W (2001). Molecular fingerprinting of bacterial population in ground water and bottled mineral water. Appl. Microbiol. Biotechnol. 57: 412-418.

Diez B, Pedros-Alio C, Marsh TL, Massana R (2001). Application of denaturing gradient gel electrophoresis (DGGE) to study the diversity of marine Pico eukaryotic assemblages and comparison of DGGE with other molecular techniques. Appl. Environ. Microb. 67: 2942-2951.

Fontana C, Vignolo G, Cocconcelli PS (2005). PCR-DGGE analysis for the identification of microbial population from Argentinian dry fermented sausage. J. Microbiol. Method. 63: 254-263.

Giovanni SJ, Britschgi TB, Moye CL, Field KG (1990). Genetic diversity in Sargasso Sea bacterioplankton. Nature, 345: 60-63.

Giraud JP (1998). Microbiologie alimentaire. Dunod/RIA. Paris, p. 696.

Heyndrickx M, Vauterin L, Vandamme P, Kerster K, De Vos P (1996). Applicability of combined amplified ribosomal DNA restriction analysis (ARDRA) patterns in bacterial phylogeny and taxonomy. J. Microbiol. Method, 26: 247-259.

Horsley RW (1977). The bacterial flora of the Atlantic salmon (*Salmo salar L*) in relation to its environment. J. Appl. Bacterial. 36: 377-386.

Jallabert B, Baroiller JF, Breton B, Fostier A, Le Gac F, Guiguem Y, Monod G (2000). Main neuro-endocrine and paracrine regulations of fish reproduction and vulnerability to xenobiotics. Ecotoxicology, 9: 25-40.

Leesing R (2005). Identification et validation des marqueurs spécifiques pour la traçabilité des poisons d'aquaculture lors de leur import/export. Thèse, Université de Montpellier II, p. 173.

Le Nguyen DD, Hanh Ha N, Dijoux D, Loiseau G, Montet D (2007). Determination of fish origin by using 16S rDNA fingerprinting of bacterial communities by PCR-DGGE: An application on Pangasus fish from Viet Nam. Food Control, 19: 454-460.

Liton J (1980). Microbiology in fishery sciences. In Connel JJ (Ed) Advances in Fishery Science and Technology. Fish News Book. Farnham, England, pp. 138-157.

Maïworé J, Tatsadjieu NL, Montet D, Loiseau G, Mbofung CMF (2009). Comparison of bacterial communities of tilapia fish from Cameroon and Vietnam using PCR-DGGE (polymerase chain reaction-denaturing gradient gel electrophoresis). Afr. J. Biotechnol. 8: 7156-7163.

Mescle JF, Zucca J (1996). Les facteurs du développement, chapitre 1. In Bourgeois CM, Mescle JF, Zucca J Ed. Microbiologie Alimentaire Tome : Aspect microbiologique de la sécurité et de la qualité des aliments, Tec et Doc-Lavoisier. pp. 3-39.

Montet D, Leesing R, Gemrot F, Loiseau G (2004). Development of an efficient method for bacterial diversity analysis: Denaturing Gradient Gel Electrophoresis (DGGE). Seminar Food Safety Int. trade, Bankok, Thaïlande.

Montet D, Le Nguyen DD, El Sheikha Aly F, Condur A, Métayer I, Loiseau G. (2008). Application of PCR-DGGE in determining food origin: Cases studies of fish and fruits. Aspects of Applied Biology 87, Greening the Food Chain, 3-4: 11-22.

Muyzer G, De Waal EC, Uitterlinden AG (1993). Profiling of complex microbial populations by Denaturing Gradient Gel Electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rDNA. Appl. Environ. Microbiol. 59: 695-700.

Muyzer G, Hottenträger Teske A, Wawer C (1996). Denaturing gradient gel electrophoresis of PCR-amplified 16S rDNA: a new approach to analyze the genetic diversity of mixed microbial communities, In Akermanns ADL, van Elsas JD and de Bruijn FJ (ed.). Molecular microbial ecology manual. Kluwer Acad. Pub. Dordrecht, Netherlands. pp. 1-23.

Ogier JC, Son O, Grus A, Tailliez P, Delacroix-Buchet A (2002). Identification of bacterial microflora in diary products by temporal Temperature Gradient Gel Electrophoresis. Appl. Environ. Microbiol. 68: 3691-3701.

Shewan JM (1962). The bacteriology of fresh and spoiling fish and some related chemical changes. In Recent advances in food science (eds Hawthorn J & Muil Leitch J). 1: 167-193.

Shewan JM (1977). The bacteriological of fresh and spoilage fish and the biochemical changes induced by bacterial action. In: Proceeding of the conference on handling processing and marketing of tropical fish, Trop. Prod. Instit. London.

Spanggaard B, Huber I, Nielsen T, Appel K, Gram L (2000). The microbiota of rainbow trout intestine: a comparison of traditional and molecular identification. Aquaculture, 182: 1-15.

Tatsadjieu NL, Maïworé J, Hadjia MB, Loiseau G, Montet D, Mbofung CMF (2009). Study of the microbial diversity of *Oreochromis niloticus* of three lakes of Cameroon by PCR-DGGE: Application to the determination of the geographical origin. Food Control, 21: 673-678.

Wilson IG (1997). Inhibition and facilitation of nucleic acid amplification. Appl. Environ. Microbiol. 63: 3741-375.

Wong HC, Chen MC, Liu SH, Liu DP (1999). Incidence of highly genetically diversified *Vibrio Parahaemoliticus* in seafood imported from Asian countries. Int. J. Food Microbiol. 52: 181-188.